

Figure 1: Various synthetic pathways for the biosynthesis of DHA (docosahexaenoic acid)

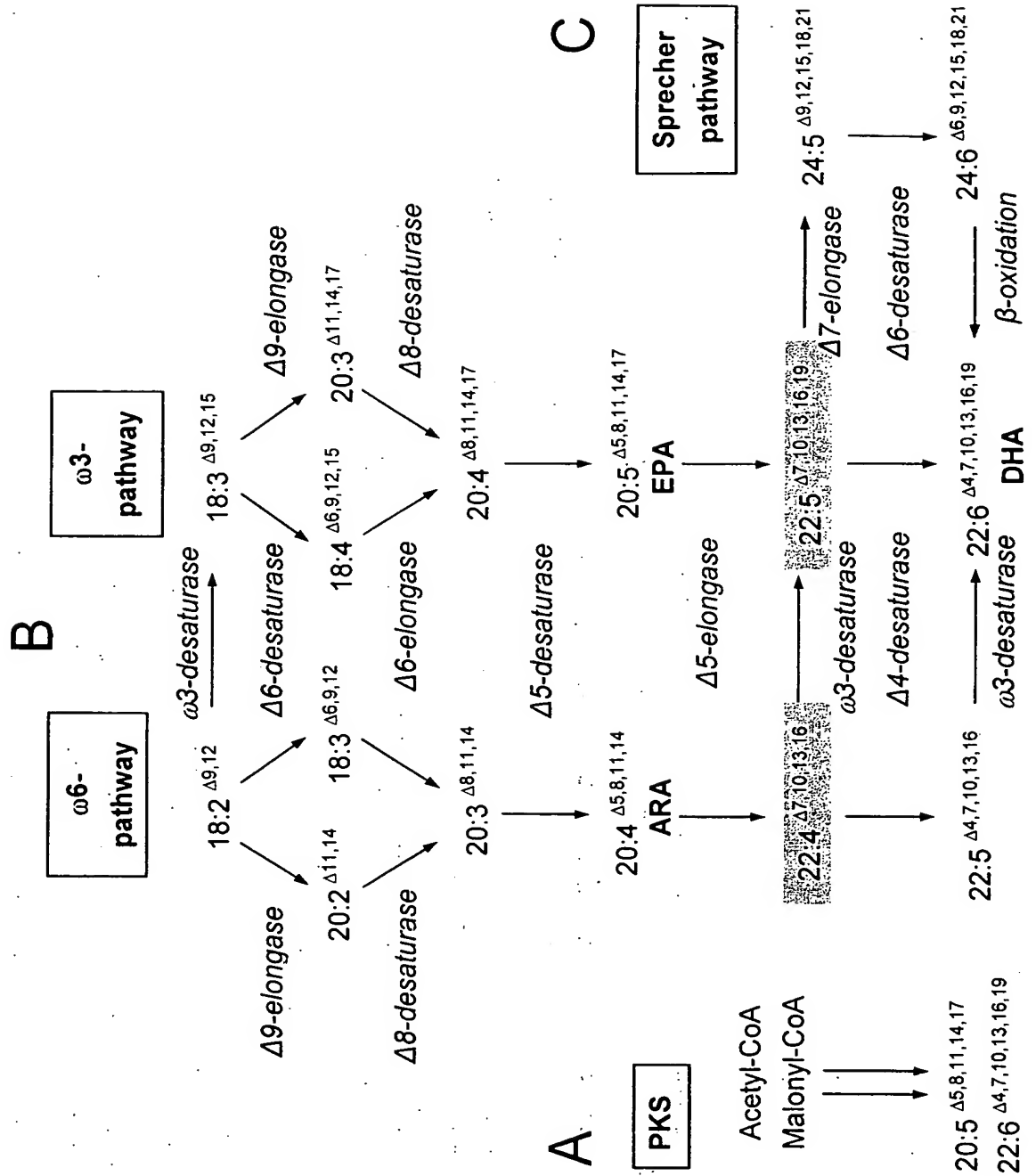


Figure 2: Sequence comparison of the $\Delta 4$ -desaturases from *Euglena gracilis* and *Thraustochytrium* (WO 200226946). The two sequences show 35% identity [CLUSTAL W(1.60) multiple sequence alignment]

<i>Euglena</i>	MLVLFGNFYVKQYSQKNGKPENGATPENGAKPQPCENGTVKEKRENDTANVRPTRPAGPPP
<i>Thrausto</i>	-----
<i>Euglena</i>	ATYYDSLAVSGQGERLFTTDEVRRHILPTDGLWLTCHGVYDVTDFLAKHPGGGVITLGL
<i>Thrausto</i>	-----MTVGDEEIPFEQVRAHNKPPDDAWCAIHGHVYDVTKFASVHPGGDIILLAA
<i>Euglena</i>	GRDCTILIESYHPAGRPDKVMEKYRIGTLQDP-----KTFYAWGESDFY
<i>Thrausto</i>	GKEATVLYETVHVGVSDAVLRKYRIGKLPDQGGGANEKEKRTL SGLSSASYYTWN SDFY
<i>Euglena</i>	PELKRRALARLKEAGQARRG--GLGVKALLVLTLLFFVSWYMWVAHKS-----FLWAAVWGF
<i>Thrausto</i>	RVMRERVVARLKERGKARRGGYELWIKAFLLLVGFWSLSLYMCTLDPSFGAILAAMSLGV
<i>Euglena</i>	AGSHVGLSIQHDGNHGAFSRNTLVNRLAGWGM DLI GASSTVWEYQHVIGHHGYTNLVS--
<i>Thrausto</i>	FAAFVGTCIQHDGNHGAFASQSRWNKVAGWTLDMIGASGMTWEFQHV LGHHPYTNLIEEE
<i>Euglena</i>	-----DTLFLSLPENDPDVFSYPLMRMHPDTAWQPHHRFQHLFAFPLFALMTIS
<i>Thrausto</i>	NGLQKVS GKKMDTKLADQESDPDVFSYPMNRLHPWHQKRWHYHRFQHIYGPPIFGFMTIN
<i>Euglena</i>	KVLTSDFAVCLSMKKGSIDCSSRLVPLEGQLLFWGAKLANFLQLIVLPCYLHGTAMGLAL
<i>Thrausto</i>	KVVTQDVGVVLRKRLFQIDAECRYASPMYVARFWIMKALT VLYMVVALPCYMQGPWHGLKL
<i>Euglena</i>	FSVAHLVSGEYLAICFIINHISECFMN-----TSFQTAARRTEM LQAAHQQA
<i>Thrausto</i>	FAIAHFTCGEVLATMFIVNHHIEGVSYSKDAVKGTMAPPKTMHGVTBPMNTRKEVEAEA
<i>Euglena</i>	AEAKVKPTPPNDWAVTQVQCVCNWRSGGVLANHL SGGNLHQIEHHLFPSISHANYPTI
<i>Thrausto</i>	SKSGAVVKSVP LDDWAVVQCQTSVNWSVGSFWNNHFSGGNLHQIEHHLFPGLSHETYHYHI
<i>Euglena</i>	APVWKEVCEEYGLPYKNVYVTFWDVAVCGMVQHRLRLMGAPPVPTNGDKKS-
<i>Thrausto</i>	QDV FQSTCAEYGVYPYQHEPSLWTAYWKMLEHLRLQLGNEETHESWQRAA-

Figure 3: Sequence comparison of the $\Delta 4$ -desaturases from *Euglena gracilis* and *Thraustochytrium* (WO 200226946).
GAP alignment

GAP Test

Gap Weight: 8 Average Match: 2.912 Length Weight: 2 Average Mismatch: -2.003
Quality: 926 Length: 590 Ratio: 1.781 Gaps: 9

Match display thresholds for the alignment(s):

| = IDENTITY; : = 2; . = 1

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51 RPTRPAGPPPATYYDSLAVSGQGKERLFTTDEVRRHILPTDGWLTCHEGV 100
      | :      :| | | | | | | |
1  .....MTVGYDEEIPFEQVRAHNKPD DAWCAIHGHV 31

101 YDVTDFLAKHPGGVITLGLGRDCTILIESYHPAGRPDKVMEKYRIGTLQ 150
      |||| | . |||| : | | :| | . | | | | :| | | | |
32 YDVTKFASVHPGGDIILLAAAGKEATVLYETYHVRGVSDAVLRKYRIGKLP 81
      . . . . .

151 DPK.....TFYAWGESDFYPELKRRLARLKEAGQARR 183
      | .      :| | | | | | :| | . | | | | | | | |
82 DGQGGANEKEKRTL SGLSSASYTW.NSDFYVMRERVVARLKERGKARR 130
      . . . . .

184 GG..LGVKALLVLT LFFVSWY.MWVAHKSF...LWAAVWGFAGSHVGLSI 227
      || :| | | . | | | | | | | | | | . | | |
  
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131 GGYELWIKAFLLLVGFWSLYWMCTLDPSFGAILAAMSLGVFAAFVGTCTI 180
228 QHDCNHGAFSRNTLVNRLAGWMDLIGASSTVWEYQHVIGHHQTNLVS. 276
|||||... ||:.||| :|:|||| ||:||||| ||||:
181 QHDCNHGAFQAQRWVNKVAGWTLDMIGASGMTWEFQHVGLGHPYTNLIEE 230
277DTLFSLPENDPDVFSSYPLMRMHPDTAWQPHHRFQHLF 314
|| : |||||||.||:|:| : ||||:|:
231 ENGLQKVSgKKMDTKLADQESDPDFSTYPMRLHPWHQKRWYHRFQHIY 280
315 AFPLFALMTISKVLTSDFAVCLSMKKGSIDCSSRLVPLEGQLLFWGAKLA 364
:| |||.||. | | : | | | | | | | |
281 GPFIFGFMtINKVVTQDVGVLKRKLFQIDAECRYASPMYVARFWIMKAL 330
365 NFLLQIVLPCYLHGtAMGLALFSVAHLVSGEYLAICFIINHISESCEFMN 414
| : |||: | || |:.|| | | | | | | | : |
331 TVLYMVALPCYMQGPWHGLKLFAIAHFTCGEVLATMFIVNHHIEGVSyas 380
415 TSF.....QTAARRTEMLQAAHQA.AEAKK...VKPTPPNDWAVTQ 452
.| | | : ||| | | . | | | | |
381 KDAVKGTMAPPKTMHGVTpMNNTRKEVEAEASKGAVKSVPLDDWAVVQ 430
453 VQCCVNRSGGVLANHLsGGLNHQIEHHLFPSISHANYPTIAPVVKEVCE 502
| ||| | || ||||| | | | | | : | | | | | | |
431 CQTSVNWsvGSWFwNHfSGGLNHQIEHHLFPGLSHETYHYHQDVfQSTCA 480

503 EYGLPYKNYVTFWDAVCGMVQHRLRLMGAPPVPTNGDKKS* 542
 |||.||.. . | | |.:||| :| . : . |
 481 EYGVVPYQHEPSLWTAYWKMLEHLRQLGNEETHESWQRAA* 520

Figure 4: GC analyses of yeast cells fed with DPA (docosapentaenoic acid). Control: yeast cells without $\Delta 4$ -desaturase [A], conversion to DHA in cells having the $\Delta 4$ -desaturase (pYES-EGD4-2) [B]

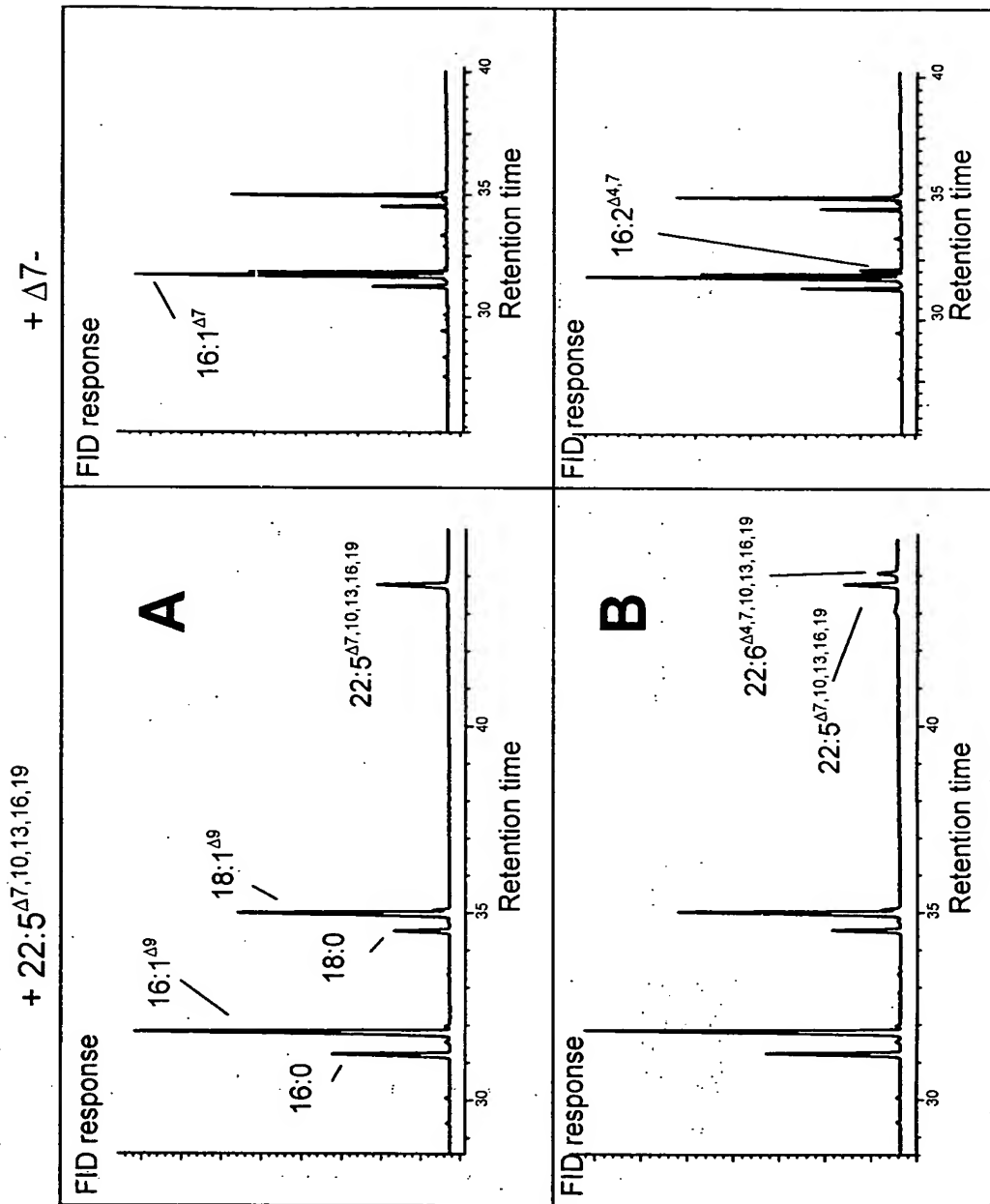


Figure 5: Position analysis of Δ^4 -desaturated fatty acids. 16:1 Δ^7 and 22:4 $\Delta^7,10,13,16$ were used for feeding. Conversion is stated in percent.

acyl group	mol % in position	
	sn-1	sn-2
16:0	31.3	4.9
16:1 Δ^9	6.5	3.5
16:2	x 4	0.6
18:0	3.6	3.3
18:1 Δ^9	2.5	11.0
18:1 Δ^{11}	0.6	1.1

acyl group	mol % in position	
	sn-1	sn-2
16:0	7	0.3
16:1 Δ^9	24.2	22.2
18:0	3.5	0
18:1 Δ^9	12.2	23.8
18:1 Δ^{11}	0.8	0.7
22:4	x 10	2.2
22:5	x 10	0.1
		0.5
		2.4